

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Before the Board of Patent Appeals and Interferences

Applicant: Yuping Ambuel Art Unit: 1651
Serial No.: 09/848,449 Examiner: Herbert J. Lilling
Filed: 05/03/2001
Title: Improved *E. coli* Extract for
Protein Synthesis
Docket No.: 700399.90126

APPELLANT'S BRIEF ON APPEAL

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Appellant, Yuping Ambuel et al., having filed a timely Notice of Appeal on September 22, 2003 in the above-identified patent application, hereby submits this brief.

I. REAL PARTY IN INTEREST

The present application is assigned to EMD Biosciences, Inc., which is a U.S. subsidiary of the German company Merck.

II. RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences.

III. STATUS OF CLAIMS

Claims 1-30 currently are pending in the subject patent application. Claims 1-9 stand finally rejected based on a final Office Action issued on June 25, 2003. A non-final Office Action was issued on November 8, 2002. Responsive remarks without any proposed amendments were filed on May 8, 2003 thereto.

As a result of a restriction requirement issued on June 26, 2002, Claims 10-30 have been withdrawn from the application and are in a non-elected status.

This appeal is specifically taken with respect to the rejected claims 1-9, which are set forth in Appendix A hereto.

IV. STATUS OF AMENDMENTS

The appellants have filed no claim amendments during the prosecution of the subject application. Appellants, however, filed a response with arguments on May 8, 2003 to the Office Action mailed on November 8, 2002.

V. SUMMARY OF THE INVENTION

The present invention as defined by the claims involved in this appeal are summarized as a preparation of a fractionated *E. coli* S-30 reaction mixture for in vitro protein synthesis, where the mixture is depleted in several macromolecules. The invention as defined by independent Claim 1 is described as a reaction mixture for performing protein synthesis reaction, the mixture includes a prokaryotic "S-30 extract" combined with a supplemental mix containing buffer, salts, nucleotide triphosphates, an energy generating system, and amino acids and depleted in Rnase E. The invention as claimed in Claim 2 provides an "S-30 extract" from *E. coli*. (See for example, the sentence bridging pages 1-2; paragraph [0005] at page 2 through paragraph [0012] at page 5; paragraph [0027] at page 6). In yet another aspect of the invention the reaction mixture is substantially removed of degradosomes. (See for example, paragraphs [0008] at pages 3-4; and paragraphs [0026], [0031], [0054], at pages 6, 8, and 15, respectively). The invention as defined by independent Claim 7 provides a reaction mixture that is fractionated by freezing, thawing and centrifugation. (See for example, paragraphs [0029-0030] at pages 7-8 and paragraph [0047] at page 13). The fractionated S-30 reaction mixture of the invention can be used to perform coupled *in vitro* transcription and translation which requires fewer pipetting steps and gives a better result than would be obtained with standard S-30 extracts and reaction procedures.

VI. ISSUES ON APPEAL

1. Claim 1-9 were rejected under 35 U.S.C. 112, first paragraph, as being unpatentable because the use of the term "S-30 extract" in the specification renders the specification a non-enabling disclosure, and therefore insufficient, because the specification fails to specify the materials which make up the "S-30 extract".

2. Claim 1-9 were rejected under 35 U.S.C. 112, second paragraph, as being unpatentable because use of the term "S-30 extract" does not specify sufficient information as to the scope of the S-30 extract.

Given these rejections, the sole issue that is being presented for review in this appeal is whether the use of the term "S-30 extract" in independent claims 1, 4, and 7 renders claims 1-9 unpatentable under 35 U.S.C. 112.

VII. GROUPING OF CLAIMS

The appellants are directing their appeal primarily toward the rejection of independent claims 1, 4, and 7. Thus, claims 1-9 stand or fall together.

VIII. ARGUMENT

The appellants submit that independent claims 1, 4, and 7, and consequently each of dependent claims 2-3, 5-6 and 8-9, are enabling based on the sufficient written description provided by the specification and thus are patentable. The appellants believe that the Examiner has chosen to refuse to accept that there is any defined meaning to the term "S-30 Extract."

In the Final Office Action to this application, the Examiner maintained the two rejections recited in the earlier non-final action. The rejections are premised under 35 U.S.C. §112, first paragraph and under 35 U.S.C. §112, second paragraph. However, both rejections are premised upon the same objection. The objection being that the term "S-30 extract" lacks sufficient definiteness. In the 112, first paragraph, rejection the Examiner contends that the use of the term "S-30 extract" in the specification renders that specification non-enabling, and therefore insufficient, because the specification fails to specify the materials which make up the S-30 extract. In the rejection under §112, second paragraph, the Examiner rejects the claims of this application on the grounds that use of the term "S-30 extract" does not specify sufficient information as to the scope of the extract.

The appellants respectfully disagree with the Examiner and traverse this rejection. While the term "extract" in and of itself may lack definiteness under some circumstances, the term "S-30 extract" is a well known and often used term of the art in the art to which this invention pertains. The art to which this invention pertains is the design and execution of systems for *in vitro* transcription and translation of genes to make proteins (e.g., cell-free protein expression). In that art, the term "S-30 extract" has a well and clearly defined meaning that is widely recognized by the art and commonly used by the workers in that field. Accordingly, Applicant is fully entitled to adopt and use a definition for "S-30 Extract" that is widely used and accepted by those practicing in the art.

An S-30 extract is no more poorly defined than any number of other mixtures, extracts and combinations used in biological systems and in patent claims. While the applicants may not be able to define all the components of an S-30 extract, no one can define all the components of a living bacterial cell, yet such cells appear often in biotech patent claims. The S-30 extract is, in fact, an extract prepared from bacterial cells through a well characterized process resulting in a product having well known properties.

At the outset, appellants assert that while the applicant in a patent application is permitted to be his own lexicographer (See, M.P.E.P. § 2173.01; *In re Hill*, 161 F.2d 367, 73 USPQ 482 (CCPA 1947)). The appellants have created new technology in this application because there was no need to do so. As demonstrated through the published citations provided hereinbelow, the appellants have used the term "S-30 extract" in a manner which is consistent with how the term "S-30 extract" is commonly known and used throughout the scientific community in referring to the prokaryotic S-30 extract.

Appellants further assert that during examination, the claims must be given their plain meaning unless an applicant has provided a clear definition in the specification. (See, *In re Zletz*, 893 F.2d 319, 321, 13 USPQ2d 1320, 1322 (Fed. Cir. 1989)). The meaning of the term "S-30 extract" was clear in the literature, so the appellants did not find it necessary to act as their own lexicographer with respect to that term. Furthermore, it is emphasized that "Claims are not to be read in a vacuum, and limitations therein are to be interpreted in light of the specification in giving them their "broadest reasonable interpretation." (See, *In re Marosi*, 710 F.2d at 802, 218 USPQ at 292 (Fed. Cir. 1983; quoting *In re Okuzawa*, 537 F.2d 545, 548, 190 USPQ 464, 466 (CCPA 1976)). Accordingly, since the appellants are fully entitled to use words in the specification that are known to those of ordinary skill in the art, there is no uncertainty or indefiniteness whatsoever in using the term "S-30 extract".

Appellants assert that the Examiner has concluded the term "S-30 extract" is indefinite only by refusing to consider the material already in the record in this patent application. First, as explained in the specification, in the sentence bridging pages 1 and 2, the use of the term S-30 extract has a clear and defining inception date. The term S-30 extract was first used in a paper by Zubay. (See, G. Zubay, "In vitro synthesis of protein in microbial systems," *Annual Review of Genetics*, 7:267-87, (1973)). A copy of that paper was supplied to the Examiner in conjunction with the IDS submitted by the appellants. In that paper, the term "S-30 extract" is repeatedly used to refer to a specific extract of materials

from bacterial cells. The paper then goes on to describe applications and uses for this type of extract.

Since, then S-30 extracts have been prepared by a variety of most modifications to the method described by the Zubay paper. At the time of filing the subject patent application, one of skill in the art could obtain S-30 extracts in either of two ways. An investigator could simply make an S-30 extract using the procedures described in, among others, the Zubay paper. Alternatively, the investigator could simply buy an aliquot of S-30 extract, sold under that name, from multiple reagent supply companies. Submitted with the IDS in this case were copies of literature describing commercial S-30 extract products from both Promega and Ambion, and these products were commercially available before the filing of this patent application. The market has clearly acknowledged certainty as to what an S-30 extract is, since both companies specifically describe their products to their customers using that term.

Likewise, the appellants have already supplied to the Examiner copies of a number of related scientific papers which explicitly use the term S-30 extract to define the materials made by the process described in the Zubay paper. In fact, the appellants have provided numerous reference materials (scientific literature, patents/published applications, websites, corporate technical bulletins, etc...) indicating that the term "S-30 extract" has a definite art-recognized meaning consistent with the scope of the present application. (*In re Barr*, 444 F.2d 588 (C.C.P.A. 1971). Thus, the appellants supplied to the Examiner clear examples of prior art references which use the term "S-30 extract", specifically with the same meaning used in the subject patent application. It is worthy of note in this regard that although the appellants have submitted a Form 1449 (filed October 2, 2001), the Examiner has not acknowledged that document or cited any of the documents supplied to the Examiner therewith.

Appellants note that several of the papers refer to the use of an S-30 extract in their very title. For example the papers to Kang¹, Lesley², and three Promega Technical Bulletins³, copies of all of which have been supplied to the Examiner in the IDS, use the term "S-30

¹ Kang, et al., "An efficient cell-free protein synthesis system using periplasmic phosphatase-removed S30 extract," *Journal of Microbiological Methods* 43:91-96 (2000).

² Lesley, S. A., "Preparation and Use of E. Coli S-30 Extracts," *Methods in Molecular Biology* 37:265-278 (1995).

³ "E. coli S30 Extract System for Circular DNA," Promega Technical Bulletin (1998); "E. coli T7 S30 Extract System for Circular DNA," Promega Technical Bulletin (1995); and "E. Coli S30 Extract System for Linear Templates," Promega Technical Bulletin (1998).

extract” in their titles. Since, according to M.P.E.P. §2111.01 the words of a claim must be given their plain meaning and read as they would be interpreted by those of ordinary skill in the art, appellants believe that these publications serve to unambiguously establish that the term “S-30 extract” is not indefinite as used in this application. (See, *In re Sneed*, 710 F.2d 1544, 218 USPQ 385 (Fed. Cir. 1983)).

The present patent application was assigned to a company known as Novagen, Inc., now a part of EMD Biosciences. Novagen offers a product made in accordance with the present invention under the trademark EcoPro. In addition to Novagen, three other manufacturers namely Promega, Ambion, and Invitrogen, offer and continue to sell S-30 extract products to the research community. All of these entities refer to these products as S-30 extracts. Description of the Promega products can be found at the following web site: http://www.promega.com/tbs/prtn_exp.htm. The product offered by Ambion is described at the following web site: http://www.ambion.com/techlib/prot/bp_1290.pdf. The product offered by Invitrogen is described at the following web site: <http://www.invitrogen.com/content.cfm?pageid=9482>. A paper describing the relative comparison of the results obtained using the process of the present invention (referred to as EcoPro, http://www.novagen.com/Products/ProductDetail_NVG.asp?catNO=70888&CatID=235) as compared to prior S-30 extract products can be found at the appellants’ web site at: http://www.novagen.com/SharedImages/TechnicalLiterature/7_nllc.pdf.

Any even cursory review of the scientific literature will reveal that the term S-30 extract is widely used in the literature to refer to a cellular extract which is exactly the same extract referred to by the appellants here. In fact, several review articles have established that *E. coli* coupled transcription and translation system, “S-30 extract”, may be prepared by using a variety of *E. coli* strains. Common strains used for preparing S-30 extracts include but are not limited to BL21, BL21DE3, JV554, D10 and SL119. One skilled in the art would certainly know what factors to consider in choosing an *E. coli* strain for preparing an “S-30 extract.” (See, Lesley, S. A., “Preparation and Use of *E. coli* S-30 Extracts,” Methods in Molecular Biology 37:265-278 (1995) also listed in the submitted Form 1449). Furthermore, it is well known that a preparation of an S-30 extract from *E. coli* contains all the enzymes and factors necessary for transcription and translation, although the extract must be supplemented with amino acids, an energy regenerating system and certain cofactors. (See, Pratt, J.M., “Coupled Transcription-Translation in Prokaryotic Cell-Free Systems,” Transcription and Translation IRL Press (1984) also listed in the submitted Form 1449).

Additionally, to emphasize the pervasive use and clear understanding of the term “S-30 extract” in the scientific literature, appellants conducted a computer search of the Scirus web site, which can be found at the URL: <http://www.scirus.com/>. A search for exact term “S30 extract” yielded 104 mentions in scientific journals and 132 mentions at web sites, and a search for “S-30 extract” yields another 40 papers and 9 websites. (The terms S-30 extract and S30 extract are used to describe the same material). Similarly, a search of the publication index at the National Library of Medicine, which can be found at the URL <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?CMD=Search&BD=PubMed>, reveals another 40 papers uncovered using the search term S30 extract and another 28 using the term S-30 extract. The web even contains a buyer’s guide comparing various extract products, referred to as S30 extracts, as they might be used in the research community. This guide can be found at <http://www.biocompare.com/molbio.asp?catid=823>.

Furthermore, to aid the Board’s understanding of the conventional meaning of the term “S-30 extract” appellants have provided hereinbelow a list of additional URLs, each of which refers to papers which can be conveniently found online in the internet, and each of which specifically references a cellular extract product by the name “S30 extract” or “S-30 extract.”

<http://www.unizh.ch/~pluckth/publications/pdf/APpub0198.pdf>,

http://216.239.33.104/search?q=cache:ruJFcg3kTC8C:www.nature.com/cgi-taf/DynaPage.taf%3Ffile%3D/nbt/journal/v19/n8/full/nbt0801_751.html+S30+extract&hl=en&ie=UTF-8,

http://www.nature.com/ncb/journal/v4/n6/fig_tab/ncb795_F5.html,

<http://www.icmask.org/icmasko5/poster/2.04.pdf>,

<http://www.bact.wisc.edu/biotech/gradstudents/JulieDavis/Profile.htm>,

<http://www.su.se/forskning/disputationer/spikblad/RichardOdegrip.html>,

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=10731664&dopt=Abstract,

<http://bletchley.tamu.edu/homepage/pdf/biophys76-1999.pdf>,

<http://www.mit.edu:8001/people/gyjung/Cell.htm>.

All of the aforementioned publications serve as suitable proofs indicating that the teaching contained in the specification is [sufficiently described] and truly enabling. (See, *In*

re Bowen, 492 F.2d 859, 861-64, 181 USPQ 48, 50-52 (CCPA 1974), specifically, section 112 requires that the scope of claims must bear a reasonable correlation to the scope of enablement provided by the specification to persons of ordinary skill in the art). Furthermore, appellants note that in *In re Bowen* the board's non-enablement rejection was reversed where the "claims literally comprehend numerous polymers in addition to the one specifically described in appellant's specification" because no persuasive reason was given by the Patent Office why the specification does not realistically enable one skilled in the art to practice the invention as broadly as it is claimed. (See, *In re Bowen*, 492 F.2d at 863, 181 USPQ at 51-52.) The same can be said here with respect to the term "S-30 extract". The only impediments are the time and cost of studies which could be performed by "those who were expert in the field and actually working" with S-30 extracts doing coupled transcription-translation experiments, i.e., those skilled in the art. Thus, each of the publications indicates that terminology such as "S-30 extract" is conventional language in this art, which apparently is well understood by those of ordinary skill in the art.

Furthermore, the USPTO's own web site, a search of issued patents revealed 142 patents using the term "S30 extract" and another 12 issued patents using the term "S-30 extract." A search of USPTO published applications revealed 220 publications using the term "S30 extract" and another 10 publications using the term "S-30 extract." These references to the term "S-30 extract" in the various scientific and patent databases serve to clearly establish that the art has adopted a definition of "S-30 Extract" which is based on an unambiguous and common understanding among those of ordinary skill in the art. It is manifestly impracticable for the Examiner to require the appellants to give an example of every component falling within the "S-30 extract". The Examiner's requirement that the applicants define every component of its claimed extract certainly has not been applied to claim reciting cells or lysates or many other cellular products. Appellants assert that it is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it. Accordingly, appellants respectfully assert the conclusion that there is nothing amiss in the form of the claim term "S-30 extract", and that one of ordinary skill in this art would be apprised of the scope of the claim in view of the terminology used in present application. See *In re Hansen*, 51 CCPA 1147, 332 F.2d 825 (1964). The term "S-30 extract" is neither vague nor indefinite.

Appellants respectfully assert that the core requirement for the applicants was to define the invention and enable the invention. Appellants believe that the core requirements

under 35 U.S.C. 112 (first and second paragraph) have been met. On the issue of enablement, the Examiner has provided no logic or reasoning why the use of the term “S-30 extract”, other than the admitted fact that the phrase includes the word “extract.” The fact that the term “S-30 (or S30) extract” is well known and used in the art to refer to a well characterized commercial product, has not been considered by the Examiner. Appellants believe that this failure to focus on the term “S-30 extract” has resulted in the Examiner’s contentions that would require the appellants to “give the exact structure of the extract or ingredients in the extract” before an appropriate search would be conducted by the Examiner. This assertion is wrong as matter of law, and as a matter of fact.

It is a requirement that the appellants define a product as completely as possible in order to clearly allow persons of ordinary skill in the art to recognize the claimed invention. (See, M.P.E.P. 2163.02; *In re Barker*, 559 F.2d 588, 592 n.4, 194 USPQ 470, 473 n.4 (CCPA 1977), cert. denied, 434 U.S. 1064 (1978)). However, it is impossible with precision to define all of the components in a material which was made from living cells. In fact, in construing claim language, the courts have recognized that in meeting the statutory disclosure requirements, it is merely necessary that the patent illustrate some embodiments of the invention and not all of them, *Ziegler v. Phillips Petroleum Co.*, 483 F.2d 858, 871 (5th Cir.), cert. denied, 414 U.S. 1079, 94 S.Ct. 597, 38 L.Ed.2d 485 (1973); *Noll v. O. M. Scott & Sons Co.*, 467 F.2d 295, 302 (6th Cir. 1972), and this view has been specifically articulated in the context of patents covering chemical processes. (See *In re Angstadt*, 537 F.2d 498, 502-03 (Cust. & Pat.App.1976); and *In re Bowen*, 492 F.2d 859, 863 (Cust. & Pat.App.1974).

The exact definition of all of the molecules which exist in a living cell is not yet known. As is clearly recited in the specification, with reference to the Zubay paper, the “S-30 extract”, as it is known in the art, is a product which is the result of a certain processing of prokaryotic cells. That processing results in an extract which contains the transcriptional and translational materials of the cells, isolated from other cellular components. This process is well understood in the technology commonly used in research labs all across the country, and taught in molecular biology courses around the United States and around the world. The resulting product is used widely in the research community and sold to that community under the name, “S-30 extract”. The material is defined in the specification to the extent it is possible to define the material given the present state of human knowledge in the present application. Thus, the apparent breadth of the term “S-30 extract” should not be equated with indefiniteness. (See, M.P.E.P. 2173.04, *In re Miller*, 441 F.2d 689, 169 USPQ 597 (CCPA

1971)). Moreover, the PTO permits claims to cells and organisms, which are complex mixtures of ingredients. There is no expressed reasoning as to why a higher standard should be applied to the subject matter of the present application.

Furthermore, the subject matter of this patent application is easily searchable, since the term S-30 (or S30) extract is commonly used for this material, as shown by the numerous search results cited above. Appellants believe that the Examiner has simply focused on the fact the name of this byproduct includes the word “extract” and implied from that that somehow this extract is indefinite in its content. As established above through the cited publications, the contrary is true. The extract is a mixture made by a defined process. The world understands how to make such an extract and use it. The literature is replete with examples of it, as referenced above. There is no uncertainty about what it means to be an “S-30 extract.”

The appellants have clearly described in its specification how a “conventional” S-30 extract may be manipulated by the processes described by the appellants in the subject application to create a variant that has improved properties and results. The appellants have characterized both the processes by which that improved extract can be made from a “conventional” S-30 extract and also has defined, to the extent it knows it, the characteristics of the improved extract which differ from a “conventional” S-30 extract. The application is thus enabling for exactly the process the appellants have described and the products the appellants claim have been defined to the extent possible.

Clearly the process for making the improved “S-30 extract” described and claimed in this patent application was enabled by this specification. For example, starting from paragraph [0035 to 0055] corresponding to pages 10-16 of the specification; appellants have provided experimental details (i.e., reagents and protocols) for making the stock solutions, the supplemental mix, culturing the cells, preparing the extract, optimizing the extract, making the bulk fractionated S-30 reaction mixture, providing the conditions for running the coupled transcription and translation protein synthesis reactions, explaining the results obtained, and characterizing the fractionated S-30 reaction mix. Thus, in its most simplistic embodiment, the improved “S-30 extract” described here can be made by taking a “conventional” S-30 extract, freezing it, thawing it, and then simply centrifuging and decanting it to make the material clear. This is recited in the specification, paragraph [0027]. Therefore, this application has provided written description of the manner and process of making and using the “S-30 extract”, in such full, clear, concise, and exact terms as to enable any person skilled

in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the appellants in carrying out the invention.

Also, since S-30 extracts were readily commercially available at the time this application was filed, and minimally competent scientist could perform this invention without any difficulty. The Examiner has provided no reasoning why there is any such difficulty. Thus, appellants assert that a case for non-enablement has not been made by the Examiner.

Again, while the appellants have correctly noted that in other context, the term "extract" in and by itself might be indefinite, that is not the case here. The combined term "S-30 extract" has a clear meaning in the art, is commonly used in the technology, and is widely referred to in the industry to refer to a specific product. There is no indefiniteness in the use of that term. The Examiner is required to acknowledge and comply with uses of the term "S-30 extract" that are consistent with how that term is commonly used by one of ordinary skill in the art. Based on the above-mentioned publications, the art of coupled transcription and translation in prokaryotic cell-free systems, the term "S-30 extract" has a clear, definite, and well understood meaning. Hence, the scope of the claimed subject matter "S-30 extract" would readily be determined by one having ordinary skill in the art. Therefore, the Examiner could exhaustively search this art, using the term "S-30 extract", if the Examiner was so inclined.

Wherefore the rejections applied by the Examiner against this patent application are respectfully traversed. Appellants, thus, respectfully request the Board to reconsider this patent application on its merits.

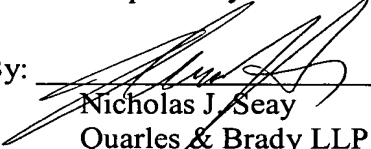
IX. CONCLUSION

In view of the above, it is respectfully requested that the rejection be overturned. The Board is hereby authorized to charge the \$165.00 appeal brief fee to Deposit Account 17-0055, together with any other fees that may be required in this appeal.

As a consequence, Appellant requests reversal of the final rejection in the instant patent application.

Respectfully submitted,

Dated: February __, 2004 By: _____


Nicholas J. Seay
Quarles & Brady LLP
1 South Pinckney Street
P O Box 2113
Madison, WI 53701-2113
TEL 608/251-5000
FAX 608/251-9166

X. APPENDIX A
Patent Application Serial No. 09/848,449
Claims Involved in the Appeal

1. A reaction mixture for performing protein synthesis reaction, the mixture comprising a prokaryotic S-30 extract combined with a supplemental mix containing buffer, salts, nucleotide triphosphates, an energy generating system, and amino acids, the reaction mixture being substantially depleted in RNase E.
2. A reaction mixture as claimed in Claim 1 wherein the extract is from *E. coli*.
3. A reaction mixture as claimed in Claim 1 wherein the mixture further comprises an amount of amino acids.
4. A reaction mixture for performing protein synthesis reactions, the mixture comprising a prokaryotic S-30 extract combined with a supplemental mix containing buffer, salts, nucleotide triphosphates, an energy generating system, the reaction mixture having the degradosomes substantially removed therefrom.
5. A reaction mixture as claimed in Claim 4 wherein the extract is from *E. coli*.
6. A reaction mixture as claimed in Claim 4 wherein the mixture further comprises an amount of amino acids.
7. A reaction mixture for performing protein synthesis reactions, the mixture comprising a prokaryotic S-30 extract combined with a supplemental mix containing buffer, salts, nucleotide triphosphates, and an energy source, wherein the reaction mixture had been fractionated by freezing, thawing and centrifugation.
8. A reaction mixture as claimed in Claim 7 wherein the extract is from *E. coli*.
9. A reaction mixture as claimed in Claim 7 wherein the mixture further comprises an amount of amino acids.